Suitably, the annealing part of the amplification is between 37°C and 65°C. The optimum temperature may be 50°C for most primer pairs, but as discussed in Example 1, a temperature of 60° C may be preferred for the Pax 2 amplification primers shown below:

Upstream primer: 5' TTTGTGAACGGCCGGCCCCTA 3' (SEQ ID NO:1)
Downstream primer: 5' CATTGTCACAGATGCCCTCGG 3' (SEQ ID NO:2)

An appealing temperature of 55° C may be preferred for the GAPDH amplification primers shown below:

Upstream primer: 5' GGCCGTATTGGGCGCCTGGTC 3' (SEQ ID NO:3)
Downstream primer: 5' GAAGGGCAACTACTGTTCGAAG 3' (SEQ ID NO:4)

These primers may be used in an amplification reaction alongside a reaction using primers, such as those described above, that may amplify nucleic acid encoding part of Pax 2, such that the GAPDH amplification that serves as a positive control for the detection of nucleic acid, as described below, particularly in Example 1.

A temperature of 72 °C may be used for the extension phase of the amplification when a thermostable polymerase is used, such as *Taq* polymerase.

Although the nucleic acid which is useful in the methods of the invention may be RNA or DNA, DNA is preferred. Although the nucleic acid which is useful in the methods of the invention may be double-stranded or

single-stranded, single-stranded nucleic acid is preferred under some circumstances such as in nucleic acid amplification reactions.

The nucleic acid which is useful in the methods of the invention may be any suitable size. However, for certain diagnostic, probing or amplifying purposes, it is preferred if the nucleic acid has fewer than 10 000, or more preferably fewer than 1000, more preferably still from 10 to 100, and in further preference from 15 to 30 base pairs (if the nucleic acid is double-stranded) or bases (if the nucleic acid is single stranded). As is described more fully below, single-stranded DNA primers, suitable for use in a polymerase chain reaction, are particularly preferred.

The nucleic acid for use in the methods of the invention is a nucleic acid capable of hybridising to the said Pax 2 mRNAs. Fragments of the said Pax 2 genes and cDNAs derivable from the mRNA encoded by the said Pax 2 genes are also preferred nucleic acids for use in the methods of the invention.

It is particularly preferred if the nucleic acid for use in the methods of the invention is an oligonucleotide primer which can be used to amplify a portion of the said Pax 2 nucleic acid, particularly Pax 2 mRNA. Examples are the primers described above:

Upstream primer: 5' TTTGTGAACGGCCGGCCCCTA 3' (SEQ ID NO:1)
Downstream primer: 5' CATTGTCACAGATGCCCTCGG 3' (SEQ ID NO:2)

The Pax 2 mRNA is similar to, but distinct from other Pax mRNAs. Preferred nucleic acids for use in the invention are those selectively hybridise to the Pax 2 mRNA and do not hybridise to other Pax mRNAs.

TTTGTGAACGGCCGGCCCCTA 3' (SEQ ID NO:1) and downstream: 5' CATTGTCACAGATGCCCTCGG 3' (SEQ ID NO:2). GAPDH (190 base pairs), upstream: 5'GGCCGTATTGGCGCCTGGTC 3' (SEQ ID NO:5) and downstream 5' GAAGGGCAACTACTGTTCGAAG 3' (SEQ ID NO:4). Negative controls may be included with water replacing cDNA.

The above primers are designed so that each anneals within an exon, but the amplified fragment crosses boundaries between exons, so that inadvertent amplification of genomic DNA would include an intron. This would easily be identified by the larger size of the fragment on the gel. Positive controls used may be cells known to express Pax 2, for example UOK 117 cells, a renal cell carcinoma cell line.

PCR products may electrophoresed on 1.8% agarose gels and transferred to nylon membranes (Qiabrane Nylon Plus, Qiagen). Filters may hybridised with a human Pax 2 cDNA probe designed from the Pax 2 cDNA sequence, for example the 1.3kb probe reference 101398 derived from the cDNA sequence from the Max Planck Institut, Goettingen, labeled with [^{32}P]dCTP using a Rediprime® random primer labelling kit (Amersham) and ExpressHybe® (Clontech) according to the manufacturer's instructions. The filters may then be exposed to x-ray film. A 20 minute exposure of the filters to the film may be sufficient for detection of the control Pax 2-containing sample and of Pax 2 positive samples, though an overnight exposure is preferred. Control GAPDH-product may be detected in an analogous manner, or by ethidium bromide detection on the gel prior to blotting or on a duplicate gel, as described below.

as for the prostate cancer samples. Fifteen radical prostatectomy specimens were obtained from patients with prostate cancer disnosed on biopsy.

PCR Amplification of Pax 2 cDNA.

Total RNA was prepared using TRIZOL® reagent (Life Technologies ™), according to the manufacturers' instructions and in each case 5 μg of total RNA was reverse transcribed using a first-strand cDNA synthesis kit (Pharmacia - protocol according to manufacturers' instructions). PCR amplification for Pax 2 cDNA was performed on the RT product. The ubiquitiously expressed GAPDH was amplified as a control. For amplification of Pax 2 cDNA, the protocol used consisted of 35 cycles of denaturation at 95 °C, annealing at 60 °C and extension at 72 °C. For GAPDH, the annealing temperature was reduced to 55 °C. Primers used and the size of PCR products were as follows: Pax 2 (301 base pairs), upstream: 5' TTTGTGAACGGCCGGCCCCTA 3' (SEQ ID NO:1) and downstream: 5' CATTGTCACAGATGCCCTCGG 3' (SEQ ID NO:2). GAPDH (190 base pairs), upstream: 5' GGCCGTATTGGCGCCTGGTC 3' (SEQ ID NO:5) and downstream 5' GAAGGGCAACTACTGTTCGAAG 3' (SEQ ID NO:4). Negative controls were included with water replacing cDNA.

All the-primers were designed so that each annealed within an axon, but the amplified fragment crossed boundaries between exons, so that inadvertent amplification of genomic DNA would include an intron. This would easily be identified by the larger size of the fragment on the gel. Positive controls used were UOK231 and UOK117 cells known to express Pax 2, PCR products were electrophoresed on 1.8% agarose gels and

transferred to nylon membranes according to the manufacturer's instructions (Qiabrane Nylon Plus, Qiagen). Filters were hybridised with